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Germination characteristics of four common perennial grasses of Inner Mongolian grassland, China

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Abstract

The grassland of Inner Mongolia, China, has experienced a decrease in area. To provide useful information for artificial grassland restoration, the germination characteristics of four native grass species, *Leymus chinensis* (Trin.) Tzvel., *Agropyron cristatum* (L.) Gaertn., *Bromus inermis* Leyss., and *Elymus dahuricus* Turcz., were compared under laboratory conditions. The seeds of *A. cristatum* and *B. inermis* showed > 70% germination at a wide range of temperatures from 10 to 30°C with or without light. Seeds of *E. dahuricus* did not show clear dormancy, but required a rather high temperature (20-30°C) for germination. When the temperature was alternated between day and night, it was the higher of the temperatures (the daytime temperature) that largely determined the percentage of germination. The germination of *E. dahuricus* was higher (max. 87%) in darkness than in light (max. 58%). Intact seeds of *L. chinensis* showed considerable germination (47%) under a wide range of temperatures (30/10°C). Additionally, the dehusking of *L. chinensis* seeds improved the germinability at a constant temperature. These results are suggestive of a different regeneration niche among the four species, which should be taken into account when selecting the species and method for grassland restoration.

Introduction

The grassland in Inner Mongolia, China, has decreased in area and deteriorated over the

past 50 years due to increased human disturbance (Meyer 2006; Ito *et al*. 2006; Akiyama and Kawamura 2007; Kawada *et al*. 2011). Current efforts, including the banning of grazing and artificial grassland restoration, have not reversed this trend (Jiang *et al*. 2006; Meyer 2006). The development of reliable technology for vegetation restoration is of primary importance in this area.

In northeastern Inner Mongolia, *Leymus chinensis* (Trin.) Tzvel. (*Aneurolepidium chinense* [Trin.] Kitag.), is the most dominant species of the typical steppe under adequate grazing pressure, and is a source of high-quality forage (Yiruhan *et al*. 2001; Liu *et al*. 2004; Kawada *et al*. 2011). Studies to improve or restore the grassland through artificial seed sowing have focused on this species. However, there are many other wild or cultivated species that could also be useful in restoration trials, including *Agropyron cristatum* (L.) Gaertn., *Bromus inermis* Leyss., and *Elymus dahuricus* Turcz. (Shao *et al*. 2006). At our study site in northeastern Inner Mongolia, *A. cristatum* and *B. inermis* are found in more heavily grazed or disturbed habitats than *L. chinensis* (Ao *et al*. 2008). In addition, *E. dahuricus* is resistant to extreme winter temperatures and adverse soil conditions (Kawada *et al*. 2011).

Knowledge of the ecophysiology in each aspect of the life cycle of the utilized species is essential for successful grassland restoration. In a previous report (Ao *et al*. 2009), we examined the underground architecture of these four species (including *L. chinensis*). All of the species are perennials, but they showed characteristic underground architectures: *A. cristatum* and *E. dahuricus* lacked rhizomes and formed separate bunches, *B. inermis* produced many short rhizomes, and *L. chinensis* produced fewer and longer rhizomes (Ao *et al*. 2009).

In this report, we describe the germination characteristics of the above four species.

Agropyron cristatum and *B. inermis* were introduced to North America in the late 19th or early 20th century, and their cultivars have been extensively used for rangeland improvement (Newell 1973; Rogler 1973). Accordingly, there are many reports on the germination characteristics of these introduced grasses in the US and Canada (Plummer 1943; Ashby and Hellmers 1955; McGinnies 1960; Smoliak and Johnston 1968; McElgunn 1974; Grilz *et al*. 1994). However, many of these materials have already been subjected to artificial selection (Smoliak and Johnston 1968), hence these results may not be applicable to wild strains in their native area. *Elymus dahuricus* and *L. chinensis* are rarely used outside their native range. In China, the poor germination of *L. chinensis* has been regarded as an obstacle to artificial stand establishment (Liu *et al*. 2004; Zhang *et al*. 2006; Ma *et al*. 2008). This low germination rate was somewhat improved in the cultivated strain, but it was accompanied by low survival rates and biomass production (Liu and Han 2008).

To develop an ecology-based strategy for grassland restoration, it is important to compare the germination traits of useful candidate species under common conditions. Here, we examined the effects of constant and alternating temperatures and light on the germination of *A. cristatum*, *B. inermis*, *E. dahuricus*, and *L. chinensis*. We also examined whether dehusking and scarification could improve the low germination rate of *L. chinensis*.

Materials and methods

Seed material

Caryopses with the lemma and palea (hereafter "seeds") of four grass species, *A. cristatum*, *B. inermis*, *E. dahuricus*, and *L. chinensis*, were collected from the grassland at Beishan Experiment Station, Hailaer (49°13′N, 119°45′E; 611 m above sea level), in the northeast region of Inner Mongolia, China, in August of 2006 and 2007. The seeds were winnowed, dried, and stored at 5°C until the four germination experiments, as described below.

Experiment 1. Germination at a constant temperature

Seeds collected in 2007 were used. The seeds were sown on 0.8% agar beds of cells in transparent plastic boxes on October 11, 2007, and kept in incubators (Type NS60; Takayama Manufacturing, Kyoto, Japan) at 5, 10, 15, 20, 25, and 30°C. One pair of boxes for each temperature was kept under a 12-h photoperiod under white fluorescent light, with the daytime light intensity set at approximately 20 μmol s^{-1} m^{-2} (400-700 nm). The other box was kept in darkness by wrapping the box with aluminum foil and two layers of silver PVC film immediately after sowing.

Experiment 2. Germination at alternating temperatures

Seeds collected in 2007 were used. The procedure was the same as in Experiment 1, except that the seeds were sown on January 8, 2008, and kept in incubators at three constant (10, 20, and 30°C) and three alternating (20/10, 30/10, and 30/20°C) temperatures (with a 12-h photoperiod), with the higher temperature corresponding to the daytime (illuminated) period for the alternating temperatures.

Experiment 3. Germinability and viability of 1-year-old and current-year seeds

Seeds collected in August 2006 and August 2007 were sown on December 7, 2007, to examine the effect of seed aging on germinability. Germination was tested at 25°C under light only. The procedure otherwise followed that of Experiment 1. Seeds remaining ungerminated on the final day of the germination test were cut longitudinally in half and subjected to the triphenyl tetrazolium chloride test, as described in McDonald and Copeland (1989) to evaluate their viability.

Experiment 4. Effect of dehusking and scarification of *Leymus* seeds

Seeds of *L. chinensis* collected in 2007 were used. A batch of dried seeds were dehusked with tweezers. The short axis bearing the lemma and palea was also removed from the caryopsis. Another batch of seeds were imbibed for 16 h at room temperature and then pierced with a thin sowing needle in the middle part of the “seed” (i.e., through the endosperm). The dehusked, scarified, and intact seeds were sown on a 0.8% agar bed of cells in transparent plastic boxes on March 12, 2008, and kept in incubators at three constant temperatures (10, 20, and 30°C) and three alternating temperatures (20/10, 30/10, and 30/20°C; 12/12 h). The procedure otherwise followed that of Experiment 1.

In all experiments, four replicates of 30 seeds were used for each condition or treatment. The test was continued for three weeks and germinated seeds were counted and removed daily. The boxes kept in darkness were opened on the final day only. Radicle emergence was used as the criterion for germination.

Statistical analysis

The germination count data were subjected to a logistic regression analysis using the glm function of the statistical software package R 2.8.0 (R Development Core Team

2008). When large overdispersion was observed, the quasibinomial error distribution was applied. When multiple comparisons were required, the data were subjected to Tukey's HSD test using the Multcomp View package in R (Graves *et al*. 2006) after arcsin transformation.

When analyzing germination at alternating temperatures (Experiment 2), logistic regression in an ANOVA-like manner was not performed because the combination between day and night temperatures was not completely factorial (i.e., the lack of combinations with higher temperatures at night). Instead, the percentage germination at each combination of day/night temperatures was arcsin-transformed and compared using Tukey's HSD test at the 5% significance level.

Results

Experiment 1. Germination at a constant temperature

Time courses of seed germination for the four grass species at six constant temperatures (5, 10, 15, 20, 25, and 30°C) under a 12-h photoperiod are shown in Figure 1. *Agropyron cristatum* and *B. inermis* started germination on the third day after sowing, and most of the germination occurred within nine days from sowing, except at the lowest temperature (5°C). The final percentage of germination was between 70 and 100% in both species (except at 5°C). In *E. dahuricus*, germination started on the fourth day after sowing at the highest temperatures (25 and 30°C) and continued through the third week, but did not exceed 60%. In *L. chinensis*, sporadic germination was observed at these constant temperatures.

The influences of temperature and light on the germination of the four species are

shown in Figure 2. A logistic regression analysis incorporating the interaction between the effects of temperature and light showed a significant interaction ($P < 0.05$) only in *A. cristatum*. Thus, the other three species were analyzed again without including the interaction term.

In *A. cristatum*, a final percentage of germination of approximately 90% was reached at 10-30°C, where no significant effect of light was observed. The germination in darkness was as low as 16.7% at 5°C. In *B. inermis*, the percentage of germination at 10-30°C ranged from 75 to 90%, but was much lower at 5°C (13.3% under a 12-h photoperiod and 0.8% in darkness). The effect of temperature was highly significant ($P < 0.001$), but the effect of light was not ($P = 0.55$). In *E. dahuricus*, the effects of temperature and light were both highly significant ($P < 0.001$) and the final percentage of germination was much higher in darkness than under a 12-h photoperiod at 15-30°C. A maximum germination rate of 86.7% was observed at 25°C in darkness. Negligible germination was observed at 5 and 10°C. The percentage of germination of *L. chinensis* was very low at all constant temperatures, regardless of the light conditions, with the maximum germination being 6.7% at 25°C under a 12-h photoperiod.

Since germination was exceptionally low at 5°C in *A. cristatum* and *B. inermis*, the data were re-analyzed at 10-30°C. The effect of light was again insignificant ($P > 0.05$) in *A. cristatum*, but the germination of *B. inermis* was significantly higher ($P < 0.001$) in darkness than under a 12-h photoperiod in this temperature range.

Experiment 2. Germination at alternating temperatures

The effects of alternating temperature on the germination of the four species are shown in Figure 3. *Agropyron cristatum* showed high rates of germination at all temperatures,

with the lowest germination being 89.3%; thus, no significant effect of alternating temperature was observed. In *B. inermis*, the percentages of germination at three alternating temperatures (95-98%) were not significantly different from those at 20 or 30°C. *Elymus dahuricus* showed the highest germination of 98.3% at the alternating temperature of 30/10°C, which was significantly higher ($P < 0.05$) than the highest value at a constant temperature (87.5% at 30°C). *Leymus chinensis* showed considerable germination only at alternating temperatures (46.7% at 30/10°C and 15.8% at 30/20°C).

Experiment 3. Germinability and viability of 1-year-old and current-year seeds

The difference in germinability between 1-year-old and current-year seeds varied between species, showing a significant interaction between species and seed age ($P < 0.01$; Figure 4). A separate analysis for each species showed significant differences between the two seed lots ($P < 0.05$) only in *A. cristatum*. The lower germination rate of the 1-year-old seeds of *A. cristatum* was due to the increase in the percentage of dead seeds; the percentage germination based on the number of viable seeds was not significantly different between the two lots ($P = 0.99$). The percentage of dead seeds was significantly higher in 1-year-old seeds than in current-year seeds for all species ($P < 0.001$).

Experiment 4. Effect of dehusking and scarification of *Leymus* seeds

The effects of dehusking and scarification on the germination of *L. chinensis* are shown in Figure 5. The effects of the seed treatments and temperature, as well as their interactions, were all significant ($P < 0.001$) according to a logistic regression analysis. The percentage germination was highest in dehusked seeds at any temperature where at

least some germination occurred. However, the differences among the seed treatments were not significant (P = 0.056) at 30/10°C where scarified and control seeds also showed high germination rates.

Discussion

Seed viability

Experiment 3 showed that the viability of current-year seeds was greater than 90% in all species (Figure 4). Thus, data correction taking seed viability into account was not required in the germination test among current-year seeds. There was a general trend towards decreased viability in 1-year-old seeds, suggesting that longer-term storage before sowing may be detrimental.

Germination characteristics of *A. cristatum* and *B. inermis*

The seeds of *A. cristatum* and *B. inermis* did not show considerable dormancy, but did show high germination rates at temperatures between 10 and 30°C with or without light (Figure 2). They likely germinate quickly when receiving sufficient amounts of rain. This is a crop-like trait that would make these species easy to use for artificial grassland restoration. Many studies conducted in North America, where introduced *A. cristatum* and *B. inermis* have been widely used for grassland establishment or improvement, reported that the seeds of these species did not show considerable dormancy and germinated under a wide range of both constant and alternating temperatures (Plummer 1943; Ashby and Hellmers 1955; McGinnies 1960; Smoliak and Johnston 1968; McElgunn 1974; Grilz *et al*. 1994). The lack of seed dormancy reported of these species

is probably not the consequence of domestication, but rather one of the reasons why they were chosen among many wild grass species.

Agropyron cristatum is a bunch-forming grass lacking rhizomes, whereas *B. inermis* is a sod-forming grass that propagates both via rhizomes and seeds (Ao *et al*. 2009). Although they showed similar germination characteristics, the role of propagation via seeds in the whole population cycle differs between these species. To adequately interpret their germination strategy, more information is required on other aspects of their life cycle (e.g., seed dispersal and seed bank dynamics).

Germination characteristics of *E. dahuricus*

The seeds of *E. dahuricus* did not show clear dormancy, but had a more specific requirement for germination than *A. cristatum* or *B. inermis*. The seeds had a rather high optimum temperature range (20-30°C) for germination (Figure 2), and if the temperature was alternated between day and night, it was the higher of the temperatures (i.e., the daytime temperature) that largely determined the percentage of germination (Figure 3). This requirement for a higher temperature may at first appear inconsistent with the low-temperature hardiness reported for this species. However, this germination requirement may be an adaptation to avoid suicidal germination late in the growing season. It is important to examine whether this species regularly forms an overwintering seed bank in the field.

The germination of *E. dahuricus* was moderately reduced in the presence of light (Figure 2). Bewley and Black (1982) and Baskin and Baskin (2001) presented numerous examples of light requirements for germination (positive photoblastism) among diverse seed plants, but few examples of germination inhibition by white light

(negative photoblastism) are available. The ecological function of negative photoblastism is not well understood (Pons 2000). Under the semi-desert climatic and edaphic environment of Inner Mongolia, seedlings germinating on the soil surface are likely prone to drought. The suppression of germination by light may also be an adaptation to reduce the risk of germinating under unsafe situations.

Germination characteristics of *L. chinensis*

Leymus chinensis showed very different germination characteristics compared to the other species. Without seed pretreatment, this species germinated only under a wide fluctuation of temperature (Figure 3). These results are in general agreement with those reported previously (Liu *et al*. 2004; Ma *et al*. 2012). *Leymus chinensis* is a dominant species of the natural grassland of this area, and forms a dense sod through its extensive rhizome system (Ao *et al*. 2009). Liu and Han (2007) experimentally showed that a vegetation gap is required for the successful emergence and establishment of *L. chinensis* seedlings, and they reported that the requirement for a fluctuating temperature for germination is a gap-detecting mechanism. Under fluctuating temperatures, the percentage germination increased gradually during the latter half of the experiment (data not shown), suggesting that the effect of temperature fluctuations may be to break dormancy rather than induce germination.

There have been contradictory reports on the effect of dehusking on seed germination in *L. chinensis*. Liu *et al*. (2004) observed no significant effect of dehusking, while Ma *et al*. (2008) reported that dehusking moderately increased both the speed and final percentage of germination. In our experiment, the dehusking of *L. chinensis* seeds considerably improved their germinability (Figure 5). Ma *et al*. (2008)

also compared germination between dehusked seeds and seeds that were replaced in the husks after dehusking, and concluded that the seed dormancy of this species is due to mechanical (but not chemical) effects of the husk. Since scarification alone did not significantly improve germination in our experiment, this “mechanical” effect is not likely due to impermeability of the seed coat. Although the exact role of the husk on seed dormancy remains unclear, these results suggest that, if a reliable method for seed dehusking could be developed, it would improve the efficiency of the artificial restoration of *L. chinensis* stands through seed sowing.

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References

Akiyama T, Kawamura K (2007) Grassland degradation in China: methods of monitoring, management and restoration. *Grassl Sci* 53: 1–17.

Ao M, Ito M, Ito K, Yun J, Miura R, Tominaga T (2008) Floristic compositions of Inner Mongolian grasslands under different land-use conditions. *Grassl. Sci*. 54: 173–178.

Ao M, Miura R, Tominaga T (2009) Root and rhizome systems of perennial grasses

grown in Inner Mongolian grassland, China. *Grassl Sci* 55: 187–192.

Ashby WC, Hellmers H (1955) Temperature requirements for germination in relation to wild-land seeding. *J Range Manag* 8: 80–83.

Baskin CC, Baskin JM (2001) *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego, 1–666.

Bewley JD, Black, M (1982) *Physiology and Biochemistry of Seeds. 2*. Springer-Verlag, Berlin. 1–375.

Graves S, Piepho HP, Selzer L (2006) multcompView: Visualizations of Paired Comparisons. (available from URL: http://www.r-project.org/)

Grilz PL, Romo JT, Young JA (1994) Comparative germination of smooth Brome and plains rough fescue. *Prairie Naturalist* 26: 157–170.

Ito M, Ao M, Ito K (2006) Inner Mongolian grasslands: situation and concern. *J Weed Sci Tech* 51: 256–262. (In Japanese)

Jiang G, Han X, Wu J. (2006) Restoration and management of the Inner Mongolia grassland require a sustainable strategy. *AMBIO* 35: 269–270.

Kawada K, Wuyunna, Nakamura T. (2011) Land degradation of abandoned croplands in the Xilingol steppe region, Inner Mongolia, China. *Grassl Sci* 57: 58–64.

Liu GS, Qi DM, Shu QY (2004) Seed germination characteristics in the perennial grass species *Leymus chinensis*. *Seed Sci Tech* 32: 717–725.

Liu GX, Han JG (2007) Influence of grassland gap on seedling establishment of *Leymus Chinensis* (Trin.) Tzvel. *Rangel Ecol Manag* 60: 624–631.

Liu GX, Han JG (2008) Seedling establishment of wild and cultivated *Leymus chinensis* (Trin.) Tzvel. under different seeding depths. *J Arid Environ* 72: 279–284.

Ma HY, Liang ZW, Wang ZC, Chen Y, Huang LH, Yang F (2008) Lemmas and

endosperms significantly inhibited germination of *Leymus chinensis* (Trin.) Tzvel. (Poaceae). *J Arid Environ* 72: 573–578.

Ma HY, Lü BS, Yang HY, Yan C, Liang ZW (2012) Responses of seed germination of *Leymus chinensis* to environmental factors in degraded grassland on Songnen Plain in China. *Chin J Pl Ecol* 36: 812–818. (In Chinese with English abstract.)

McDonald MB, Copeland LO (1989) *Seed Science and Technology Laboratory Manual*. Iowa State University Press, Ames, Iowa, 1–231.

McElgunn JD (1974) Germination response of forage grasses to constant and alternating temperatures. *Can J Pl Sci* 54: 265–270.

McGinnies WJ (1960) Effects of moisture stress and temperature on germination of six range grasses. *Agron J* 52: 159–162.

Meyer N (2006) Desertification and restoration of grasslands in Inner Mongolia. *J Forestry* 104: 328–331.

Newell LC (1973) Smooth bromegrass. In: *Forages: The Science of Grassland Agriculture 3rd edition* (Eds Heath ME, Metcalfe DS, Barnes RE), Iowa Univ. Press, Ames, Iowa, USA, 254–262.

Plummer AP (1943) The germination and early seedling development of twelve range grasses. *Agron J*, 35: 19–34.

Pons TL (2000) Seed responses to light. In: *Seeds: the Ecology of Regeneration in Plant Communities. 2nd Edition* (Ed. Fenner M), CABI Publishing, Oxon, 237–260.

R Development Core Team (2008). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, available from URL: http://www.r-project.org/ [accessed 2012].

Rogler GA (1973) The wheatgrasses. In: *Forages: The Science of Grassland Agriculture*

3rd edition (Eds Heath ME, Metcalfe DS, Barnes RE), Iowa Univ. Press, Ames, Iowa, USA, 221–230.

Shao XQ, Wang K, Dong SK, Huang XX, Kang MY (2006) Regionalisation of suitable herbages for grassland reconstruction in agro-pastoral transition zone of northern China. *N Z J Agric Res* 49: 73–84.

Smoliak S, Johnston A (1968) Germination and early growth of grasses at four root-zone temperatures. *Can J Pl Sci* 48: 119–127.

Yiruhan, Hayashi I, Nakamura T, Shiyomi M (2001) Changes in floristic composition of grasslands according to grazing intensity in Inner Mongolia, China. *J Japan Grassl Sci* 47: 362–369.

Zhang WD, Bi JJ, Ning TY, Liu XL, He MR (2006) Effects of temperature, light and other treatments on seed germination of *Leymus chinensis*. *Can J Plant Sci* 86: 143–148.

Figure legends

Figure 1 Time course of germination of four grass species at six constant temperatures.

Figure 2 Germination of the seeds of four grass species as affected by temperature and light. Error bars, shown only where larger than the symbols, indicate standard errors of the mean.

Figure 3 Germination of four grass species under constant and alternating temperatures. In each species, columns with different letters are significantly different according to Tukey's HSD test ($P < 0.05$).

Figure 4 Percentages of germinated, dormant, and dead seeds among 1-year-old (2006) and current-year (2007) seeds of four grass species.

Figure 5 Effect of dehusking and scarification on the germination of *Leymus chinensis* seeds. Error bars indicate standard errors of the mean.

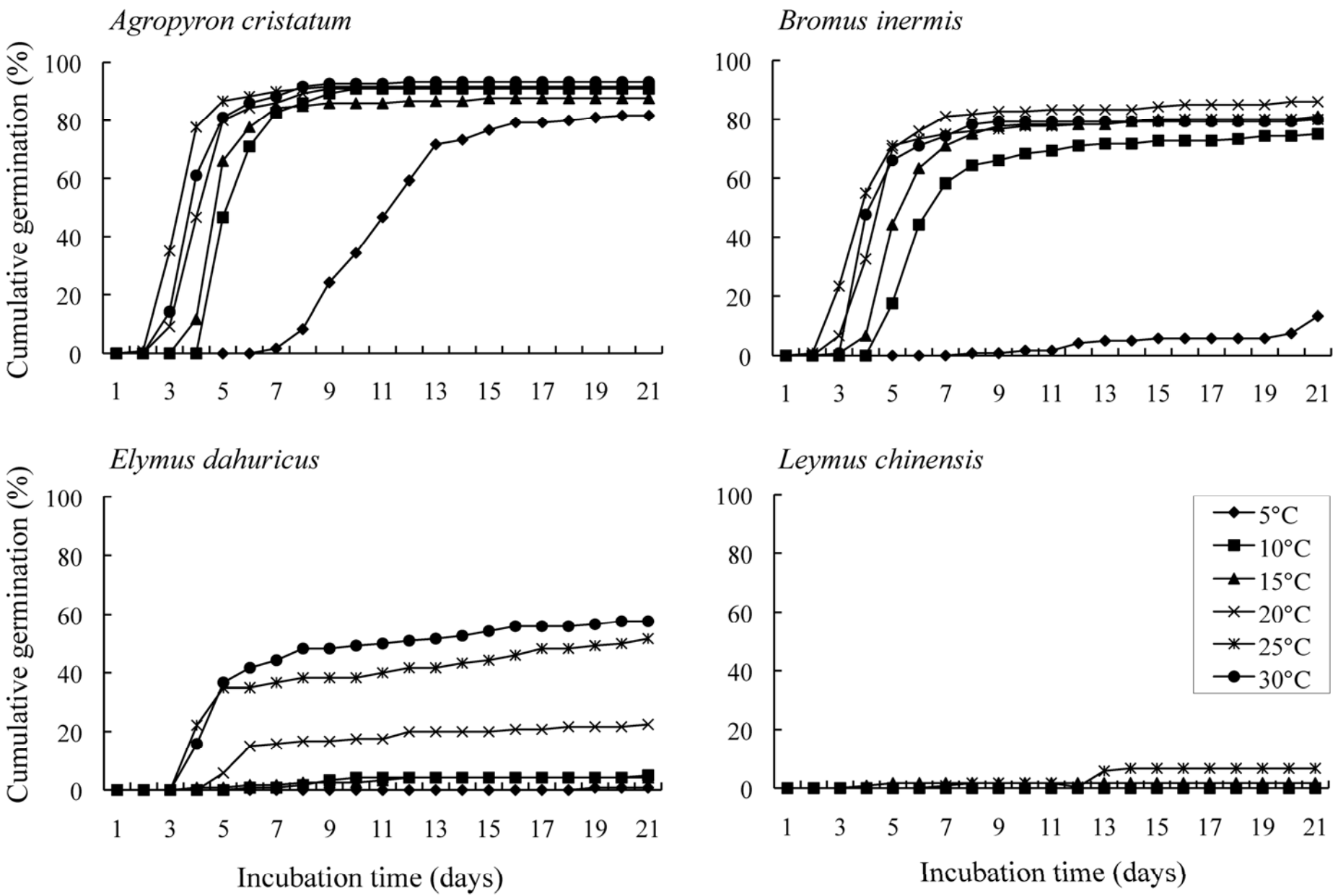
Agropyron cristatum
Bromus inermis
Elymus dahuricus
Leymus chinensis
Cumulative germination (%)
Incubation time (days)
0
20
40
60
80
100
1
3
5
7
9
11
13
15
17
19
21
5°C
10°C
15°C
20°C
25°C
30°C

Figure 1

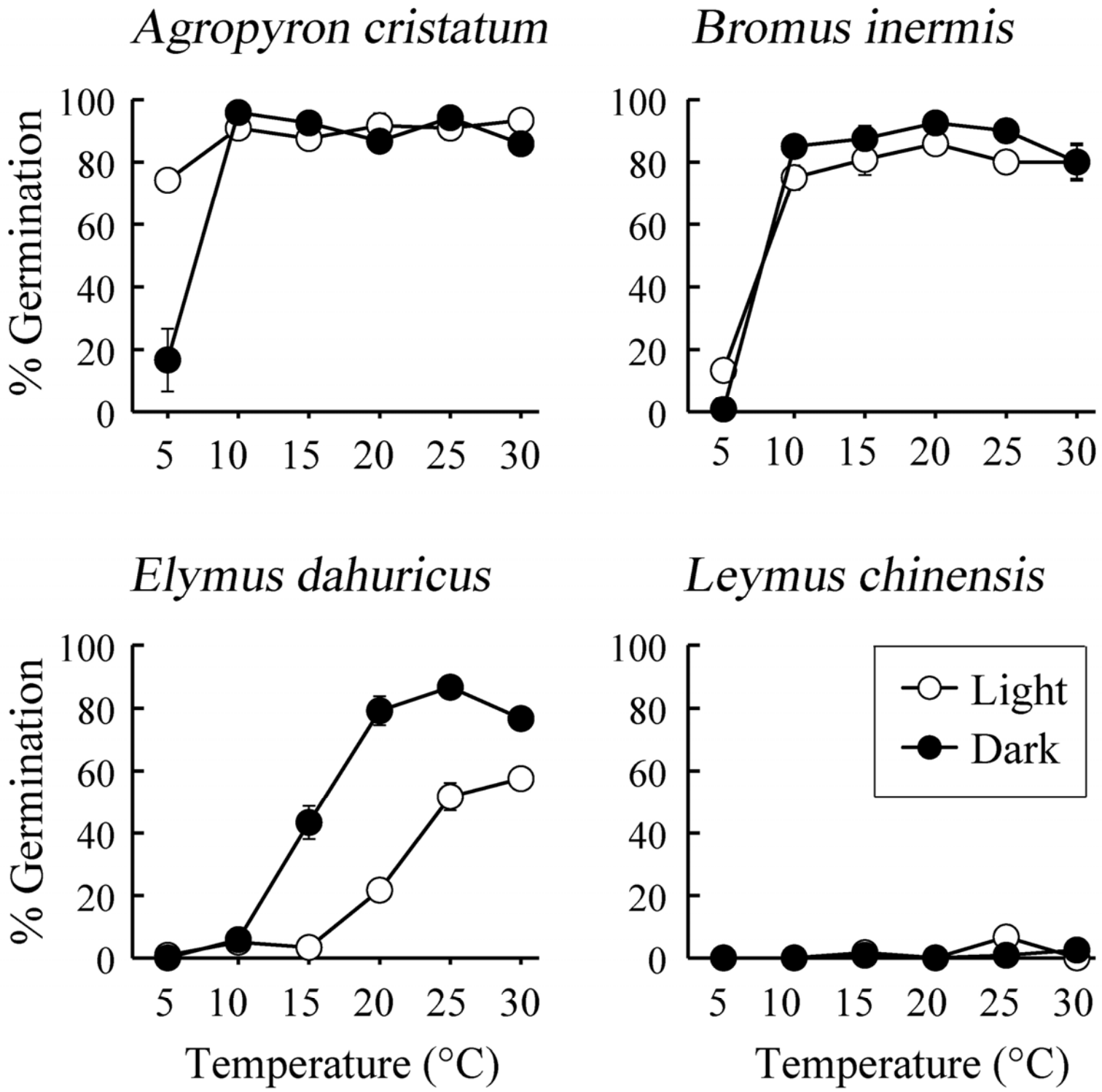
Agropyron cristatum
Bromus inermis
Elymus dahuricus
Leymus chinensis
% Germination
Temperature (°C)
Light
Dark
0
20
40
60
80
100
5
10
15
20
25
30

Figure 2

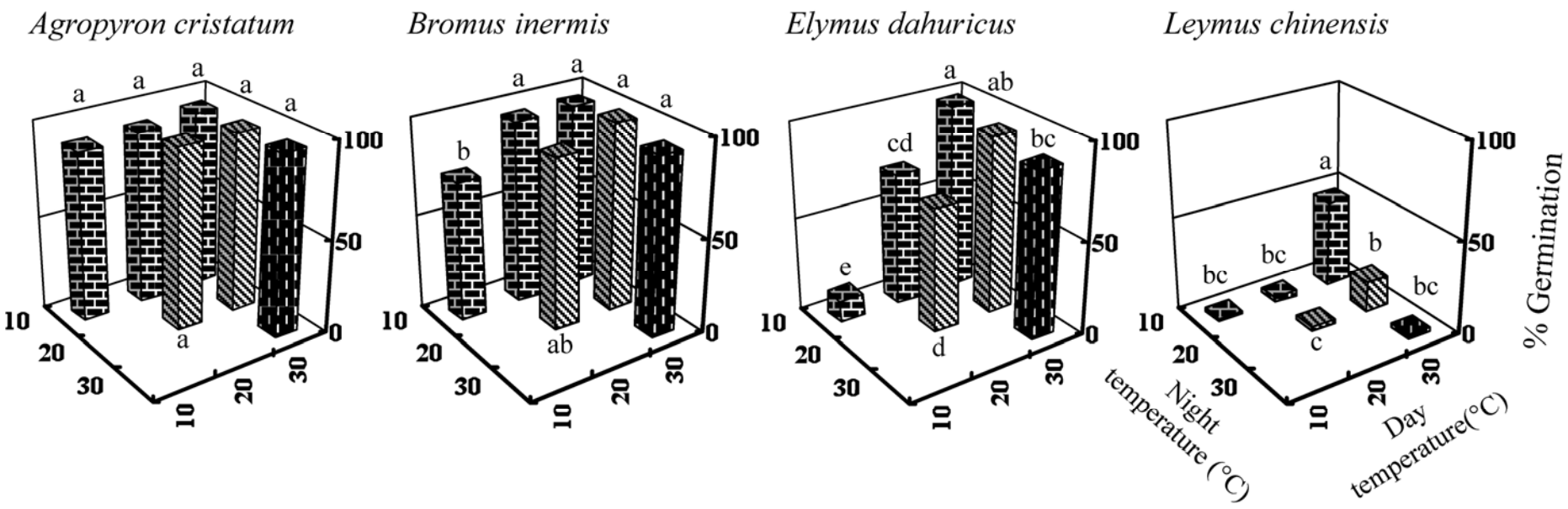
Agropyron cristatum
Bromus inermis
Elymus dahuricus
Leymus chinensis
% Germination
Night temperature (°C)
Day temperature(°C)
10
20
30
0
50
100

Figure 3

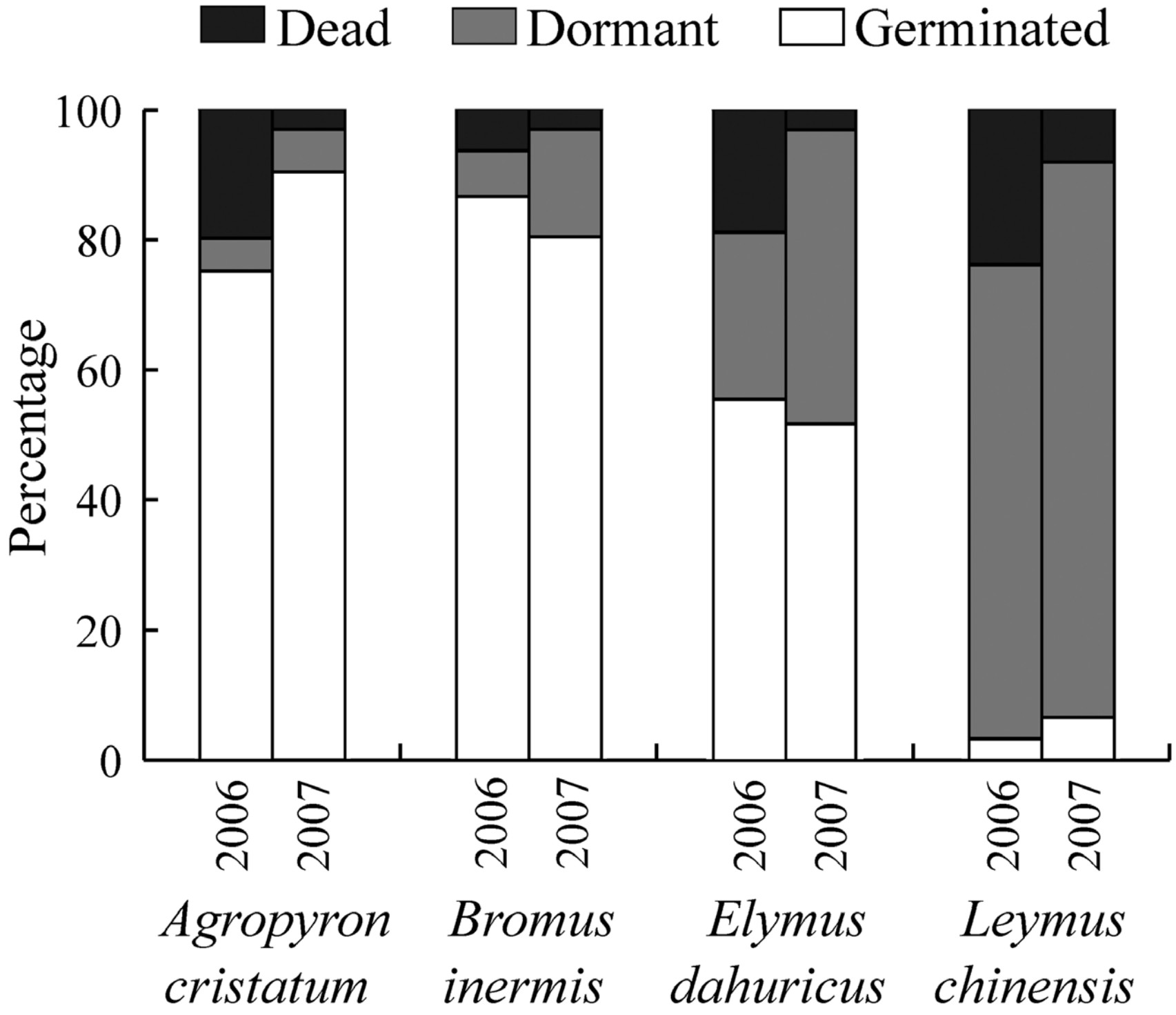
Dead
Dormant
Germinated
Percentage
100
80
60
40
20
0
2006
2007
2006
2007
2006
2007
2006
2007
Agropyron cristatum
Bromus inermis
Elymus dahuricus
Leymus chinensis

Figure 4

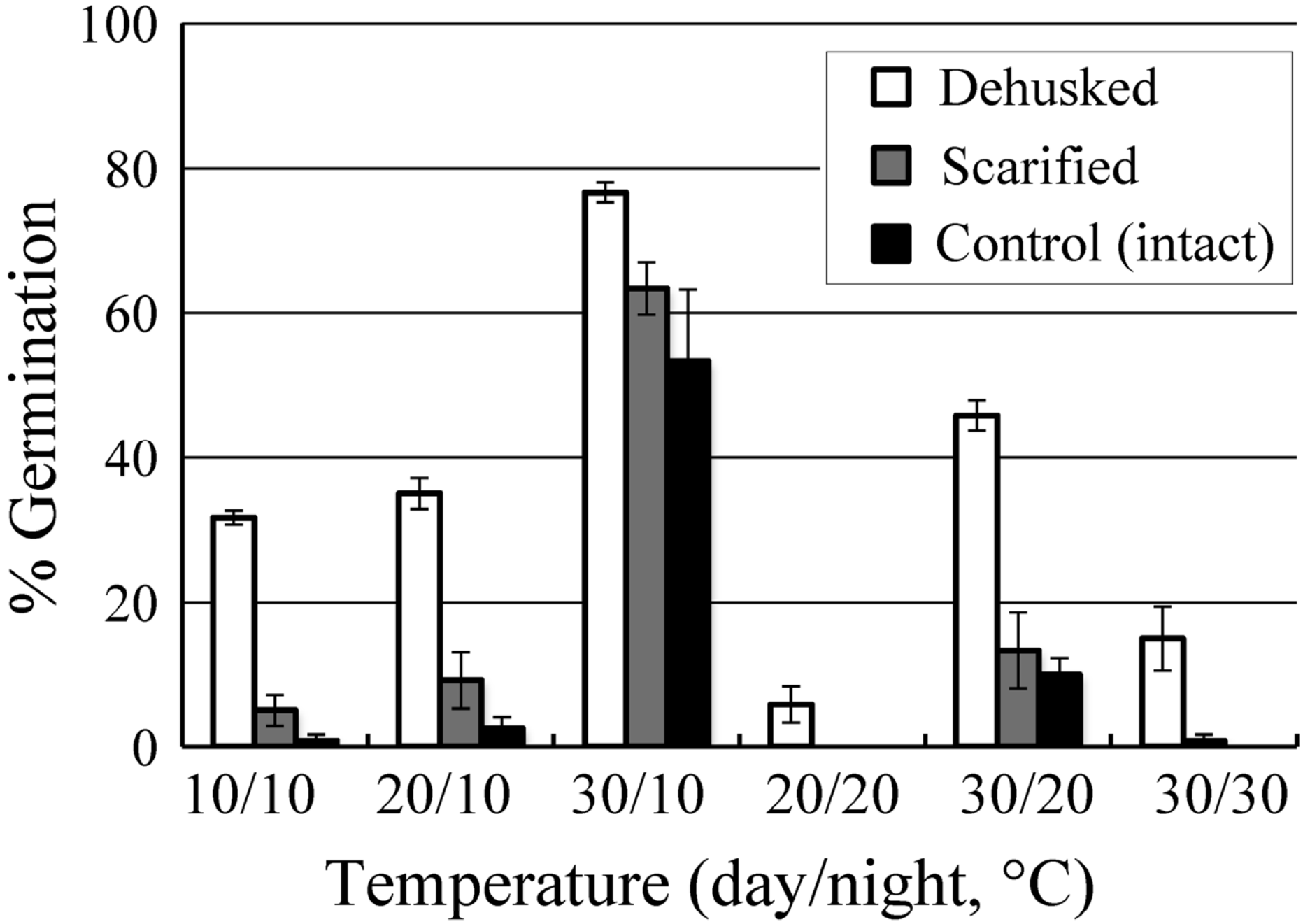
100
80
60
40
20
0
% Germination
Dehusked
Scarified
Control (intact)
10/10
20/10
30/10
20/20
30/20
30/30
Temperature (day/night, °C)

Figure 5